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No Evidence for an Allelic Association Between Schizophrenia and Markers D22S278 and D22S283

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We report a case control association study using markers D22S278 and D22S283 in 90 unrelated patients with DSMIII-R schizophrenia and 90 controls matched for ethnicity, age and sex. No differences between allele frequencies for either marker were observed when the two groups were compared (D22S278: $\chi^2 = 6.53$, df = 7, $P = 0.51$; D22S283: $\chi^2 = 14.73$, df = 15, $P = 0.48$). These findings fail to support previous work by others suggesting the presence of allelic association between the markers D22S278 and D22S283 and schizophrenia. ***Am. J. Med. Genet. 74:37–39, 1997.*** **© 1997 Wiley-Liss, Inc.**

KEY WORDS: schizophrenia; allelic association; DNA markers; chromosome 22

INTRODUCTION

Several studies have suggested linkage between genetic markers on the long arm of chromosome 22 and schizophrenia [Pulver et al., 1994a; Coon et al., 1994], though negative findings have also been reported [Pulver et al., 1994b].

Recently Vallada et al. [1995a] examined chromosome 22 using 14 polymorphic markers in 23 multiplex pedigrees. Although none of the markers yielded significant lod scores, D22S278 and the nearby marker D22S283 showed excess allele sharing by affected siblings ($P < 0.01$). These data were combined with those from 10 other groups in a meta-analysis of D22S278, which confirmed significant allele sharing in affected sib-pairs ($P = 0.001$), suggesting the presence of a schizophrenia susceptibility locus in this region of chromosome 22 [Schizophrenia Collaborative Linkage Group on Chromosome 22, 1996]. Subsequently both D22S278 and D22S283 were shown to demonstrate significant transmission disequilibrium [Vallada et al., 1995b]. A separate haplotype relative risk study of 113 Chinese trios has also revealed a possible allelic association between the locus D22S278 and schizophrenia [Moises et al., 1995]. If replicated, these findings would suggest the presence of a susceptibility locus in very close proximity to D22S278 and D22S283.

In view of this we have performed a case control association study using markers D22S278 and D22S283 in a sample of schizophrenic patients and matched controls. Our results have also been directly related to those of Vallada et al. [1995b] by examining the frequency in probands and controls of alleles 146 bp and 148 bp for D22S283, and of alleles 231 bp and 237 bp for D22S278.

MATERIALS AND METHODS

Ninety unrelated patients with schizophrenia (57 males and 33 females) were studied. These included one affected member, chosen at random prior to genotyping, from 13 families containing several affected members and 52 sibships containing two or more affected siblings. The remaining 25 subjects were recruited from local inpatient and day hospital facilities. Ninety unrelated controls (57 males and 33 females) were recruited from a local branch of the National Blood Transfusion Service (Wales). The mean ages of the two groups were: patients 44.7 years (SD = 13.5) and controls 44.2 years (SD = 12.3).

An Operational Criteria Checklist [OPCRIT 3.31; McGuffin et al., 1991] was completed for each patient on the basis of a semistructured interview [PSE 9; Wing et al., 1974; or SCAN; Wing et al., 1990] and all available clinical information. All patients satisfied the DSMIII-R criteria for schizophrenia [American Psychiatric Association, 1987]. Controls were matched to patients on the basis of sex, age (±5 years), and ethnicity (i.e., Welsh, Irish, and English). All the patients and controls were Caucasians born in the UK or Ireland. In each case the parents were also born in the UK or Ireland. Of the patients and their matched controls, 52 were born in South Wales and also had both parents born in South Wales. The controls were not specifically screened for schizophrenia, although it should be noted

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that the policy of the Blood Transfusion Service is to take donations only from unmedicated, well individuals. Written informed consent was obtained from both patients and controls after procedures had been fully explained.

High-molecular-weight DNA was isolated from lymphocytes according to routine procedures. PCR was performed in a 12.5 µl reaction containing 30 ng of genomic DNA, 12.5 pmol of each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) 200 µM dNTPs, and 1 unit *Taq* polymerase (Amersham Life Sciences). The cycling conditions for D22S278 and D22S283 were 94°C for 5 mins followed by 30 cycles of 56 and 54°C, respectively, followed by extension at 72°C and denaturation at 94°C each for 30 secs. The final extension step was at 72°C for 10 mins. PCR products were resolved on 6% denaturing polyacrylamide gels and then autoradiographed for 24 hr. Genotypes were scored independently by 2 investigators blind to clinical diagnosis.

The presence of an allelic association was sought for using CLUMP [Sham et al., 1995]. The chi-square goodness of fit test was used to test for association between schizophrenia and the frequencies of those alleles showing preferential transmission in the study of Vallada et al. [1995b]. These were alleles 146 bp and 148 bp for D22S283 (alleles 6 and 5 of Vallada et al.), and of alleles 231 bp and 237 bp for D22S278 (alleles 6 and 3 of Vallada et al.).

RESULTS

Genotypes were obtained from 86 individuals for D22S278 and 89 for D22S283. Eighty-five subjects were typed for both markers. Allele frequencies in probands and controls for both of the markers tested are shown in Tables I and II. No significant difference was found for either marker D22S278 ($\chi^2 = 6.53$, df = 7, $P = 0.51$) or D22S283 ($\chi^2 = 14.73$, df = 15, $P = 0.48$).

No significant differences were observed in the frequencies of alleles 146 bp and 148 bp between patients and controls for D22S283 ($\chi^2 = 2.56$, df = 1, $P = 0.22$; odds ratio = 0.65, 95% confidence interval = 0.38–1.10), and of alleles 231 bp and 237 bp for D22S278 ($\chi^2 = 1.25$, df = 1, $P = 0.92$; odds ratio = 1.03, 95% confidence interval = 0.66–1.59).

DISCUSSION

We observed no difference in the allele frequencies of D22S278 and D22S283 in our sample of 90 schizophrenic patients and 90 matched controls and there is no trend in our data to suggest allelic association between either marker and schizophrenia.

Our results conflict with the previous findings of allelic association between markers D22S278 and D22S283 and schizophrenia. Vallada et al. [1995b] demonstrated significant transmission disequilibrium in a set of large families. We failed to replicate these findings for both markers. In the case of D22S283 this is unlikely to be due to inadequate power as when assuming an α value of 0.05 and 1 df, our sample of 178 individuals had 98% power to detect the moderate effect observed by Vallada et al. ($w = 0.33$) [Cohen et al., 1988]. The weaker effect detected in marker D22S278 ($w = 0.08$) [Cohen et al., 1988] was more likely to have been missed as our sample had only 27% power to detect such an effect, assuming $\alpha = 0.05$ and 1 df. The use of TDT in families already shown to demonstrate linkage is questionable as the transmission of alleles within the pedigrees is then not mutually independent. It therefore remains possible that the findings by Vallada et al. [1995b] are, in fact, due to linkage. Furthermore the weak allelic association detected by Moises et al. [1995] was not statistically significant after correction for multiple testing an may well have represented a type I error.

It is unlikely that we have failed to demonstrate an association due to population stratification in our sample, because both patients and controls were closely matched for ethnicity, sex, and age.

In conclusion, we have failed to find allelic association between schizophrenia and markers D22S278 and D22S283. However, given the evidence for linkage at 22q12–13 it would be prudent to continue testing for allelic association using other markers from this region.

TABLE I. Distribution of Alleles at Marker D22S278

	Alleles (bp)							
	229	231	233	235	237	239	241	243
Affecteds	1	4	19	44	60	36	7	1
Controls	0	3	22	55	60	23	6	3

TABLE II. Distribution of Alleles at Marker D22S283

	Alleles (bp)							
	124	128	130	132	134	136	138	140
Affecteds	0	2	10	27	16	7	21	15
Controls	1	3	6	18	25	8	14	8
	Alleles (bp)							
	142	144	146	148	150	152	154	156
Affecteds	20	19	15	14	7	2	2	1
Controls	27	17	23	18	4	4	1	1

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